Analysis of DNA and Protein Adducts of Benzo[a]pyrene in Human Tissues Using Structure-Specific Methods: A Literature Review

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Goal

The aim of this review is to summarize the current literature, focusing on selective methods for the measurement of BPDE-DNA and BPDE-protein adducts and their association to various exposures.

Introduction

Benzo[*a*]pyrene (B*a*P) is an ubiquitous pollutant that is metabolically activated to benzo[*a*]pyrene-7,8-diol-9,10-epoxide (BPDE). BPDE reacts with DNA, producing a major adduct at the N^2 position of deoxyguanosine (BPDE-N^2-dG). Research clearly documents the presence of this adduct in target tissues of animals treated with B[*a*]P. The mutagenicity of BPDE-N^2-dG and its effects on DNA conformation have also been conclusively demonstrated. ^{32}P-Postlabeling and ELISA have been widely used for the detection of "PAH adducts" in human, but they provide no structural information. Despite the low limit of detection, a structure specific analysis can not be made. In recent years structure specific techniques such as HPLC-fluorescence, GC-MS and LC-LIF have became available. These techniques have been applied to the analysis of BPDE adducts in DNA and protein.

Methods

Publications regarding B[*a*]P adducts have been obtained from PubMed search. Included were studies analyzing BPDE-adducts in human specimens with structure specific detection, that have been published before January 2001. These searches gave 25 papers analyzing a total of 659 DNA and 886 protein samples for BPDE- adducts.

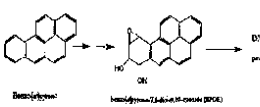

Figure 1. Benzo[a]pyrene is metabolically activated to the benzo[a]pyrene-7,8-diol-9,10-epoxide that binds to DNA and protein.

BPDE-DNA adducts

Table I: BPDE-DNA adducts in human lung analyzed by structure-specific methods

Method	Subjects	No samples / No analyzed (%)	Adducts (10^8 nucleotides) Mean ± SD	Range[a]	LOD	References
HPLC-fluo	Autopsy	4/91 (4)	1.02 ± 1.15[b]	0.09 – 1.95	1	(Tsuruta, et al., 1991)
HPLC-fluo	Autopsy	5/23 (22)	5.2 ± 4.8[b]	1.2 – 11.8	1	(Randerath, et al., 1993)
HPLC-fluo	Lung cancer patients	4/17 (24)	10.1 ± 11.1[b]	2 – 26	0.66	(Andersson, L. et al., 1996)
HPLC-fluo	Lung cancer patients	11/17 (65)	17.9 ± 12.1[b]	2 – 38	0.66	(Andersson, L. et al., 1996)
HPLC-FD	Lung cancer patients (Kiruna)	Smokers Ex-smokers Non-smokers	9/11 (82) 3/5 (100) 0/1 (0)	2.7±3.1[b]	0.5 – 9.9 3.3-23.1	0.2[c] (Alexandrov, K. et al., 1992)
HPLC-FD	Lung cancer patients (Kiruna)	Smokers Ex-smokers Non-smokers Unknown	6/26 (92) 0/14 (0) 0/6 (0) 0/1 (0)	1.83 ± 2.29 ND ND ND	0.1 – 8.4	0.2[c] (Bryant, M. et al., 1988)
HPLC-FD	Autopsy Child	Non-smokers Ex-smokers Smokers	11/21 (52) 6/6 (100) 12/13 (92)	3.76 ± 3.69 4.54 ± 5.27 5.64 ± 2.26	0.21 – 9.1 0.68 – 9.1 0.27 – 19.9	0.2[c] (Godschalk, M. et al., 1998)
HPLC-FD	Lung cancer patients (Minnesota)	Smokers Ex-smokers Non-smokers Unknown	0/9 (0) 3/10 (30) 1/13 (8) 0/1 (0)	ND		1[d]

[a] Ranges given, or data for individual samples.
[b] Calculated from corresponding fold-increase, assuming non detected samples = one-half the detection limit.
[c] Reported detection limit.
[d] Sample size was 100 µg DNA.
LOD: limit of detection; ND: not detected.

Table 2: BPDE-DNA adducts detected in different tissues analyzed by structure-specific methods

Method	Tissue	No samples / No analyzed (%)	Adducts (10^8 nucleotides) Mean ± SD	Range[a]	LOD	References
HPLC-FD and HPLC-MS	Placenta	10/10 (100)			N.A.	(Manchester, D.K. et al., 1989)
GC-MS	Urothelial cells of tumor Smokers Non-smokers	3/3 (100) 7/8 (87)	9.5 ± 8.08 1.9 ± 2.27	3.1 – 44.7 0.9 – 6.1	1[b]	(Veldhuizen, A.M. et al., 1999a)
32P-Post	Arterial synovial tissue Smokers Non-smokers	6/6 (100) 3/3 (100)	2.8 ± 0.86 1.44 ± 1.1	1.8 – 3.6 1.0 – 2.5	1[b]	(Randerath, et al., 1997a)
HPLC-FD, EtBr-FD	Atherosclerotic lesions	7/7 (100) 3/3 (100)	4.93 ± 0.89[c] 0.5 ± 0.06[c]	3.8 – 5.7 0.5 – 1.5	1[b]	(Randerath, et al., 1997)
HPLC-FD	Colon	4/7 (57)	0.5 ± 0.06[c]	0.5 – 1.5	1[b]	(Alexandrov, K. et al., 1996)
HPLC-FD	Pancreas	0/5 (0)		ND	1[b]	(Alexandrov, K. et al., 1996)

[a] Ranges given, or data for individual samples.
[b] Calculated from corresponding molar relationship, assuming non detected samples = one-half the detection limit.
[c] Sample size was 100-500 µg DNA.
[d] Sample size was 1 mg.
LOD: limit of detection; N.A. not available.

Table 3: BPDE-DNA adducts in human blood cell DNA analyzed by immunoassay methods

Method	Subjects	No samples / No analyzed (%)	Adducts (10^8 nucleotides) Mean ± SD	Range	LOD	References
GC-MS	Lung cancer patients (Sweden)	10/10 (100)	Median = 0.07	(0.07 – 11.2)[a]	N.A.	(Pastorelli, R. et al., 1998)
HPLC-FD	Lung cancer patients Healthy abattoirs, blue workers	6/7 (86) 3/3 (100)	23.5 ± 19.9[b] 8.7 ± 7.1[b]	4.9 – 55.2[b] 4 – 19[b]	N.A.	(Rojas, M. et al., 1995)
HPLC-Flu	Coke oven workers Control subjects	20/20 (100) 7/10 (70)	18.7 ± 19.6 2.0 ± 1.7	1.8 – 71.8 0.4 – 5.2	1[b]	(Rojas, M. et al., 1995)
HPLC-FD	Coke oven workers (Sweden) GSTM1 active GSTM1 "0/0"	11/20 (55) 6/9 (75) 11/11 (100)	ND 3.42 ± 3.8	0.2 – 9.4	0.2[c]	(Hayes, M. et al., 1998)
HPLC-FD	Coke oven workers Power plant workers (Sweden) GSTM1 "0/0" GSTM1 active	10/20 (50) 20/46 (54) 0/20 20/26	Median = 2.8 Median = 0.4 ND Median = 1.4	2.4 – 4.1 0.2 – 5.1 0.2 – 4.4	0.1[c]	(Hayes, M. et al., 2000)
HPLC-FD	Control subjects Arcelor-Mittal steel workers Cleaning workers Coke oven workers Foundry patients	8/24 (33) 4/12 (33) 4/18 (22) 12/16 (75) 2/16 (13)	3.8 ± 3.9 11.5 ± 14.3 4.2 – 8.1 18.2 ± 20.3 2.3 ± 1.4	ND – 10.2 ND – 36.9 ND – 53.6 ND – 72.4 ND – 4.8	2[c]	(Pavanello, S. et al., 1999)

[a] Estimated from figure 1 as reference (Pastorelli, R. et al., 1998).
[b] Calculated from corresponding fold-increase, assuming non detected samples = one-half the detection limit.
[c] Sample size was 100 µg DNA.
LOD: limit of detection; N.A. not available.

BPDE-protein adducts

Table 4: BPDE-protein adduct levels in occupationally exposed humans analyzed by several biomarker methods

Method	Subjects	Exposure Specification	Source[a]	No samples / No analyzed (%)	Adducts (fmol/mg protein) Mean ± SD	Range	LOD	References
HPLC-FD	Foundry workers	47.6 PAH/µg[b] 65.6 PAH/µg[b]	Hb	9/9 (100) 9/9 (100)	31.3 ± 3.4 31.8 ± 2.1	26 – 36 27 – 35.3	N.A.	(Carmichael, N. et al., 1999)
HPLC-FD	Creosote workers Control	All Non-smokers Smokers Non-smokers Smokers	BA	19/20 (95) 9/9 (100) 10/11 (91)	2.57 2.71 5.52 10.3	0.9 – 5.9 1.2 – 4.2 2.2 – 10.3	1[c]	(Tang, F. et al., 1996)
GC-MS	Garage mechanics Control subjects		Hb	15 14			0.2[c]	(Veldhuizen, A.M. et al., 1999b)
GC-MS	Coke oven workers Control subjects		BA	15/15 (100) 13/15 (87)			0.2[c]	(Veldhuizen, A.M. et al., 1999b)

[a] Hb, hemoglobin; BA, serum albumin.
[b] Adduct values are expressed as fmol BP tetraols/mg protein.
[c] Reported detection limit.
[d] Calculated from corresponding molar relationship, assuming non detected samples = one-half the detection limit.
[e] Sample size was 1 mg protein.
[f] Sample size was 10 mg.
LOD: limit of detection; N.A. not available.

Table 5: Protein adduct levels in occupationally exposed humans analyzed by several biomarker methods

Method	Subjects	Exposure Specification	Source[a]	No samples / No analyzed (%)	Adducts (fmol/mg protein) Mean ± SD	Range	LOD	References
GC-MS	Aluminum workers (Milan, Italy)	1,3[b] Smokers (3) Non-smokers	Hb				0.1[c]	(Pavanello, S. et al., 1996)
GC-MS	Coke oven workers Controls (Milan, Italy)	Smoker Smoker	Hb	9/9 (100) 8/10 (80)			0.05[c]	(Pavanello, S. et al., 2000)
GC-MS	Coke oven workers	Smokers	BA	16/16 (100)		105 – 0.51	0.05[c]	(Pavanello, S. et al., 2000)
GC-MS	Lung cancer patients	Smokers	Hb	6/6 (100) Smoker (100)		90 – 2.5[d] 47 – 0.5[d]	0.05[c] type	(Pavanello, S. et al., 1997)
HPLC-FD & GC-MS	Volunteers	All Smokers Non-smokers Passive smokers	BA	26/39 (67) NA/21 NA/18 NA/22		NA	0.02[c]	(Tuntaki, A. et al., 1998)
HPLC-FD & GC-MS	Volunteers	Smokers All non-smokers	Hb	23/27 (100) 19/52 (96)			0.005[c]	(Scherer, G. et al., 2000)
		Smokers All non-smokers	BA	19/52 (63) 23/52 (81)		36 – 0.004 ND – 0.192	0.01[c]	
GC-MS	Unspecified	Smokers Non-smokers	Hb	16/16 16/16 (100)		1.2 – 9.3 0.7 – 1.3	0.2	(Veldhuizen, A.M. et al., 1997)
HPLC-FD & GC-MS	Workers' wives/ parents		BA	69/69 (95)	0.32	<0.04 – 0.77	0.04	(Scherer, G. et al., 2000)

[a] Hb, hemoglobin; BA, serum albumin.
[b] Adduct levels are expressed as 2nd BP tetraols/mg protein.
[c] Reported detection limit.
[d] Calculated from corresponding molar relationship, assuming non detected samples = one-half the detection limit.
[e] Sample size was 100-200 mg.
[f] Sample size was 10 mg.
LOD: limit of detection; ND: not detected.

Summary Tables

Table 6: Samples with detectable BPDE-DNA adducts [% (n)][a]

BPDE-DNA	Exposure[b]						GSTM1[c]	No known	Total[d]
Source	Occupational	Environmental	Non-smokers	Former smokers	Smokers	% "0"	Active	exposure	
Lung			6/9 (29)	5/9 (15)	17/9 (81)	100% (4)	8/11 (1)	3/5 (43)	48% (57)
Immune tissue				1/1 (50)	2/3 (50)		7/7 (54)	1/1 (50)	
Blood cell DNA	26% (21)	43% (31)	40% (20)	29% (14)	49% (37)	50% (28)	47% (19)	n.a.	40% (169)
Total[d]	26% (21)	30% (37)	29% (29)	28% (29)	49% (40)	49% (32)	49% (30)	42% (43)	40% (169)

[a] The limits of detection ranged from 0.2–2 adducts/ 10^8 nucleotides depending on amount of DNA available (0.05–1 mg).
[b] Grouped by exposure independent from other exposures.
[c] GSTM1 genotype subgroups of main study.
[d] Columns add to total for rows do not, because some subjects are part of multiple groups.

Table 7: Samples with detectable BPDE-protein adducts [% (n)][a]

BPDE-Hb-adducts	Exposure[b]				Total[c]
Source	Occupational	Environmental	Non-smokers	Smokers	
Hemoglobin	100% (16)	43% (133)	7% (90)	36% (16)	40% (254)
Serum Albumin	57% (7)	54% (13)	20% (20)	64% (11)	40% (51)
Total[c]	96% (23)	50% (146)[d]	42% (110)	57% (27)	39% (305)

[a] Limit of detection ranged from 0.005 to 0.2 depending on the amount of protein (range from 10–200 mg) used for the analysis.
[b] Grouped by exposure independent from other exposures.
[c] Columns add to total for rows do not, because some subjects are part of multiple groups.
[d] Excluding the ILSI study (Scherer, G. et al., 2000) reduces the percentages slightly (40% versus 39% of 424 samples and 43% versus 44% in total versus smokers (see text for details)

Table 8: BPDE adduct levels associated with various exposures

Exposure	BPDE-DNA in lung	BPDE-DNA in blood	BPDE-protein
Tobacco	+	+	+
Occupational	n.d.	+	+
Environmental	n.d.	n.d.	+

+ = clearly higher in exposed individuals
n.d. = insufficient data

Conclusions

The presence of BaP adducts cannot be assumed, even in situations where exposure to BaP is relatively high, when applying the structure-specific methods available today.

Results

In Table 1-3, we present the studies on BPDE-DNA adducts. The majority of detected BPDE-DNA adduct levels ranged from 0.5 – 10 adduct/ 10^8 nucleotides, applying methods with limits of detection of 1 – 2 BPDE adducts/ 10^8 nucleotides. The detectability ranges form 40 to 56% in samples obtained from lung, somatic tissues or WBC, as summarized in Table 6. There was no single exposure situation that led to an overwhelming presence of detectable adducts. DNA adducts of BaP were detected in 50% of smokers, 46% of former smokers, 52% of non-smokers, 39% of occupationally exposed individuals, and 33% of environmentally exposed people.

In Table 4 & 5, we present the studies on BPDE-protein adducts. The majority of detected BPDE-protein adduct levels ranged from 0.1 – 200 fmol BPDE/ mg protein, applying methods with limits of detection of 0.05 – 0.2 fmol BPDE/ mg protein. Detectability ranged from 46 to 59% in the samples. Similar to the results on BPDE-DNA adducts, no single exposure was associated with higher BPDE-protein adducts levels or higher frequency of positive detected samples. Protein adducts of BaP were detected in 59% of smokers, 65% of non-smokers and 50% of environmentally exposed people. Only two studies reported data on occupational BPDE-protein adducts, one with only 15 subjects and another that reported 76% of samples with positive detected adducts for both control and exposed workers.

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